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# Range extension and new records of two Oxyuroidea Cobbold, 1864 parasitizing Cliff Chipmunk, *Neotamias dorsalis* (Baird, 1855), in the Área de Protección de Flora y Fauna Cerro del Mohinora, Chihuahua, México

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**Abstract.** As part of an inventory of the parasitic helminths of rodents, two species of the class Oxyuroidea (*Heteroxynema cucullatum* Hall, 1916 and *Rauschtineria eutamii* (Tiner, 1948) Hugot, 1980) were collected from three Cliff Chipmunk specimens (*Neotamias dorsalis* (Baird, 1955)) in the Área de Protección de Flora y Fauna Cerro del Mohinora, Mexico. These nematodes have been recorded in different species of the genus *Neotamias* (Illiger, 1811), which can be found in sympatry mainly along the entire west coast of the USA. This is the first record of both species in Mexico.

**Key words.** Geographic distribution, helminths, *Heteroxynema cucullatum*, mammals, Nematoda, parasites, *Rauschtineria eutamii*

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## INTRODUCTION

The Área de Protección de Flora y Fauna Cerro del Mohinora (ÁPFF) is in the municipality of Guadalupe y Calvo, at the southwestern tip of the state of Chihuahua (Mexico), near the borders with the states of Sinaloa and Durango. Covering an area of 9,126 ha and reaching an elevation of 3,300 m above sea level, the ÁPFF is at the highest area in Chihuahua and the entire Sierra Madre Occidental. The area boasts a great variety of ecosystems, including undisturbed old coniferous forests, representing one of the richest biodiversity-rich pine and pine-oak forest ecosystems in the state. Additionally, there are coniferous forests of *Picea* Mill., *Abies* Mill., and *Pseudotsuga* Carrière, featuring species typical of boreal habitats (SEMARNAT 2017).

Sixty-five species of wildlife have been recorded in the area, including mammals (24 spp.), birds (20 spp.), reptiles (10 spp.), amphibians (nine spp.), and fish (two spp.) (SEMARNAT 2017). In the ÁPFF, five species of rodents belonging to the family Cricetidae (*Microtus mexicanus* (Saussure, 1861), *Peromyscus boylii* (Baird, 1855), *P. gratus* Merriam, 1898, *P. maniculatus* (Wagner, 1845), and *P. melanotis* J.A. Allen & Chapman, 1897) were trapped, and they were found to be parasitized by five species of nematodes (*Aspiculuris americana* Erickson, 1938, *Pterygodermatites* (*Paucipectines*) *peromysci* Lichtenfels, 1970, *Syphacia* sp., *S. peromysci* Harkema, 1936, and *Trichuris* sp.) (Preisser and Falcón-Ordaz 2019). In the present work, we contribute to the knowledge of parasitic nematode diversity of the wild fauna in the ÁPFF.

## METHODS

We collected parasites from three individuals of *Neotamias dorsalis* Baird, 1955, Cliff Chipmunk (Mammalia, Rodentia, Sciuridae). Our fieldwork was carried out in ÁPFF in October and November 2017, using methods of trapping and handling mammals approved by the American Society of Mammalogists (Kelt et al. 2010; Sikes and the Animal Care and Use Committee of the American Society of Mammalogists 2016). *Neotamias*

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*dorsalis* were collected with a permit granted to J. A. Fernández (FAUT-036) issued by SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales). Specimens are deposited in the Colección de Mamíferos de la Facultad de Zootecnia y Ecología de la Universidad Autónoma de Chihuahua (UACH-M). Nematodes collected are deposited in the Colección Nacional de Helmintos (CNHE) del Instituto de Biología, Universidad Nacional Autónoma de México. The collected specimens were sacrificed, and their viscera were removed by placing them in 70% alcohol or 4% formalin until they were reviewed. The nematodes obtained were stored in 70% alcohol and cleared with a mixture of glycerin and 70% alcohol (2:1) for identification. Photographs and measurements were made using a Zeiss microscope equipped with an Axiocam 5RCs. Taxonomic identification was corroborated by comparing morphological traits with specialized literature: Quentin (1975) and Hugot (1980). Taxonomically important measurements are in micrometers (µm), unless otherwise indicated; the range is followed by the mean and standard deviation (Tables 1, 2). The classification used for nematodes is that proposed by Hodda (2022).

## RESULTS

Phylum Nematoda Cobb, 1932
Superfamily Oxyuroidea Cobbold, 1864
Family Heteroxynematidae Skryabin & Schikhobalova, 1948
Genus *Heteroxynema* Hall, 1916

### *Heteroxynema cucullatum* Hall, 1916

Figure 1A–D

**New record.** MÉXICO – Chihuahua • ÁPFF, Cerro del Mohinora, Guadalupe y Calvo; 25°57′40.4″N, 107°02′21.9″W; 3062 m alt.; 14.X.2017; Jesús A. Fernández leg.; intestinal caecum; 12 ♂, 3 ♀, CNHE 10921.

**Identification.** Our specimens of *H. cucullatum* are characterized by having, in both sexes, two prominent cervical alae that end at the esophagus–intestine junction (Figure 1A). These are continued with a small lateral alae, which cannot be seen where they end (Figure 1B). The male has a cuticular crest in its caudal

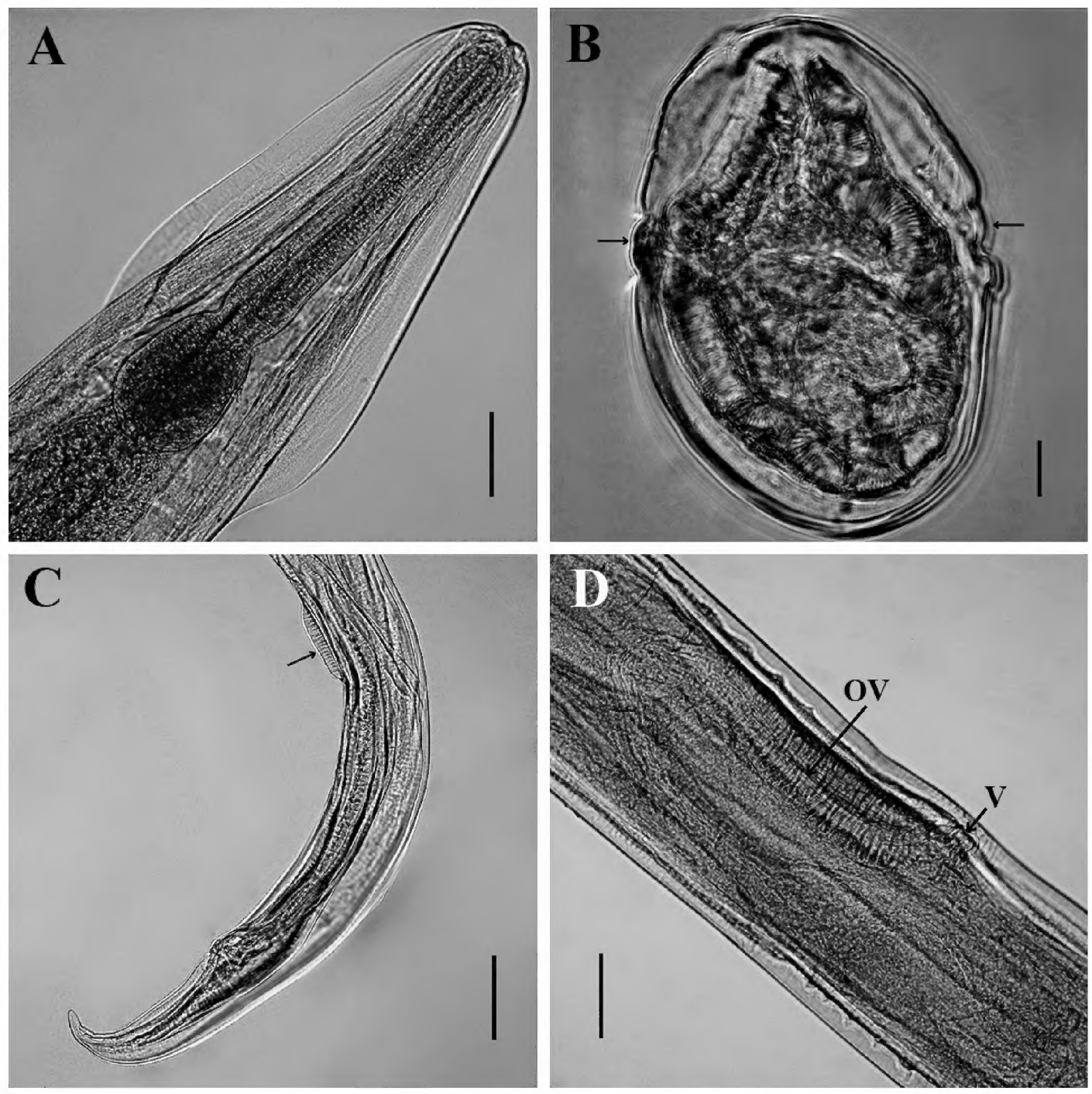


**Figure 1.** Optical microphotographs of *Heteroxynema cucullatum* showing external morphology. **A.** Anterior end of a male showing cervical alae that ends at the esophagus–intestine junction. **B.** Cross section at mid-body of a female, showing lateral alae (arrows). **C.** Posterior end of male showing the cuticular crest (arrow). **D.** Location of vulva (V) and ovijector (OV) in a female in lateral view. Scale bars: A, C, D = 100 µm; B = 20 µm.

region (Figure 1C), and do not have spicules. The vulva of the female opens in front of the middle of the body, and the ovijector is highly muscular (Figure 1D). Measurements of the specimens are presented in Table 1.

Family Oxyuridae Cobbold, 1864
Genus *Rauschtineria* Hugot, 1980

***Rauschtineria eutamii* (Tiner, 1948) Hugot, 1980**
Figure 2A–F

**New record.** MÉXICO – CHIHUAHUA • ÁPFF, Cerro del Mohinora, Guadalupe y Calvo; 25°57′41.8″N, 107°02′13.8″W; 3079 m alt.; 4.XI.2017; Jesús A. Fernández leg.; intestinal caecum; 6 ♂, 11 ♀, CNHE 10906.

**Identification.** Our specimens of *R. eutamii* are characterized by the presence of a cephalic inflation, small cuticular alae that run throughout the body and cephalic papillae approximately equidistant from each other (Figure 2A, C, D). Males have two cuticular crests behind the middle of the body (Figure 2B). The cephalic region has three simple lips, with three prominent teeth in the females (Figure 2E). The male exhibits a

**Table 1.** Measurements of *Heteroxynema cucullatum* sampled from *Neotamias dorsalis* from Área de Protección de Flora y Fauna Cerro del Mohinora, and comparison with other records of *H. cucullatum* parasitizing other species of *Neotamias*. Measurements are presented as the range, with mean and standard deviation in parentheses.

| **Host** | ***N. amoenus*** | | ***N. minimus*** | ***N. palmeri*** | ***N. dorsalis*** | |
|---|---|---|---|---|---|---|
| | Hall 1916 | | Quentin 1975 | Quentin 1975 | This study | |
| Sex, number | ♂ | ♀ | ♂, *n* = 2 | ♀, *n* = 3 | ♂, *n* = 12 | ♀, *n* = 3 |
| Total length (mm) | 2.78–4.47 | 7.39–7.9 | 7.25 | 8.2 | 2.7–4.1 (3.6 ± 0.5) | 6.5–9.1 (7.8 ± 1.3) |
| Length esophagus with bulb (µm) | 491–687 | 628–775 | 900 | | 450–550 (507 ± 26) | 635–660 (645 ± 13) |
| Cervical alae length (µm) | | | 650 | 500–600 | 390–540 (464 ± 52) | 565–575 (568 ± 6) |
| Length of cuticular mamelon (µm) | 112–130 | | 130 | | 80–115 (97 ± 13) | |
| Distance of cuticular mamelon (µm) | 515–600* | | 600† | | 380–740* (583 ± 96) | |
| Vulva (mm) | | 1.8–3.26 | | 3.15 | | 2.48–3.22 (3.00 ± 0.40) |

*Distance to tail.
†Distance to cloaca.

**Table 2.** Measurements of *Rauschtineria eutamii* sampled from *Neotamias dorsalis* from Área de Protección de Flora y Fauna Cerro del Mohinora, and comparison with other records of *R. eutamii* parasitizing other species of *Neotamias*. Measurements are presented as the range, with mean and standard deviation in parentheses.

| **Host** | ***N. minimus*** | | ***N. amoenus*** | | ***N. dorsalis*** | |
|---|---|---|---|---|---|---|
| | **Tiner 1948** | | **Hugot 1980** | | **This study** | |
| Sex, number | ♂ | ♀ | ♂, *n* = 9 | ♀, *n* = 14 | ♂, *n* = 6 | ♀, *n* = 11 |
| Total length (mm) | 2.07–2.56 | 6.4–9.9 | 2.58 | 7.65 | 2.0–2.3 (2.1 ± 0.1) | 3.3–9.7 (4.4 ± 2.1) |
| Length esophagus with bulb (µm) | 244–348 | 472–538 | 320 | | 265–285 (279 ± 8) | 325–450 (402 ± 36) |
| Length of cephalic inflation (µm) | | 300 | 190 | 120 | 125–210 (171 ± 28) | 82.5–400 (208.2 ± 92) |
| Cuticular mamelons | | | | | | |
| Anterior mamelon length (µm) | 99–165 | | 125 | | 70–75 (71 ± 2) | |
| Length of anterior region (µm) | | | 1200 | | 960–1140 (1065 ± 85) | |
| Length posterior region (µm) | 798–1150 | | | | | |
| Posterior mamelon length (µm) | 98–165 | | 130 | | 75–80 (78 ± 3) | |
| Length of anterior region (µm) | | | 1670 | | 1200–1530 (1370 ± 165) | |
| Length of posterior region (µm) | 539–660 | | | | | |
| Spicule | 72–92 | | 104 | | 125 | |
| Gubernaculum | 21–26 | | 22 | | 20 | |
| Vulva (mm) | | 0.74–1.22 | | 1.07 | | 0.57–1.28 (0.90 ± 0.24) |

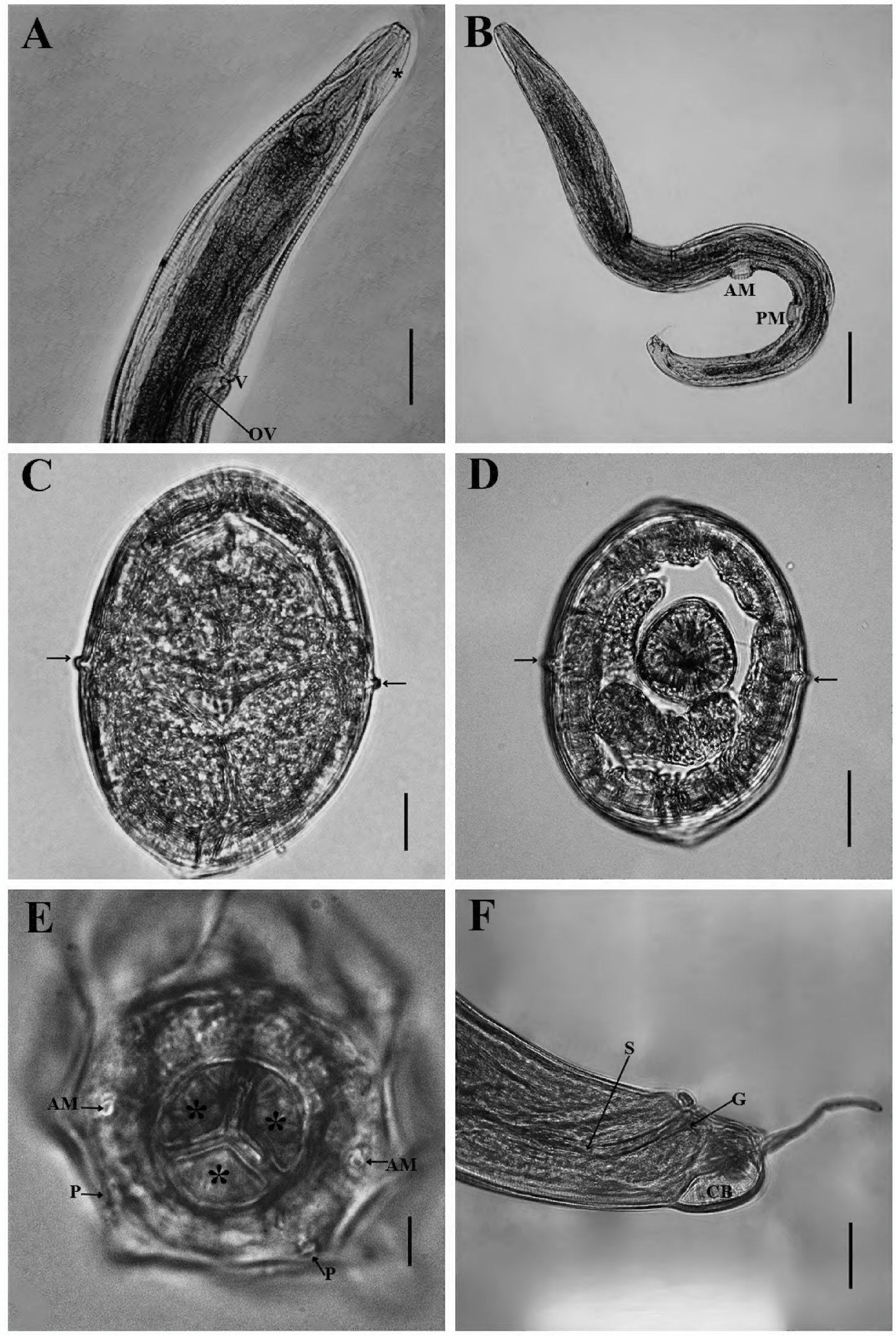


**Figure 2.** Optical microphotographs of *Rauschtineria eutamii* showing external morphology. **A.** Anterior end of the female showing the cephalic inflation (arrow), and the location of the vulva (V) and ovijector (OV) in a female in lateral view. **B.** Total view of a male in lateral view showing two cuticular crest anterior (AM) and posterior crest (PM). **C.** Cross section in at mid-body of a male, showing the lateral alae (arrows). **D.** Cross section in the esophagus of a female, showing the lateral alae (arrows). **E.** Mouth apical view of a female showing the three prominent teeth (*),papillas (P) and amphids (AM). **F.** Posterior end of male shows the tail relatively short caudal bursa (CB), spicule (S), and gubernaculum (G). Scale bars: A, B = 200 µm; C = 2 µm; D = 5 µm; E = 1 µm; F = 50 µm.

relatively short tail, showing a caudal bursa, and the spicule and gubernaculum was only observed in one male (Figure 2F). Females have the vulva located in the anterior region close to the excretory pore. Measurements of the specimens are presented in Table 2.

## DISCUSSION

The two nematode species collected belong to separate families: *Heteroxynema cucullatum* to Heteroxynematidae and *Rauschtineria eutamii* to Oxyuridae. These organisms have only been found in chipmunks, and our specimens exhibit the same morphological traits as previously described. Measurements of *H. cucullatum* fall within the ranges mentioned by Hall (1916) and Quentin (1975) (Table 1). For the specimens of *R. eutamii*, we observed variation in the measurements of the mamelons and spicule in males. The mamelons are smaller than previously documented, and the spicule is larger (Table 2).

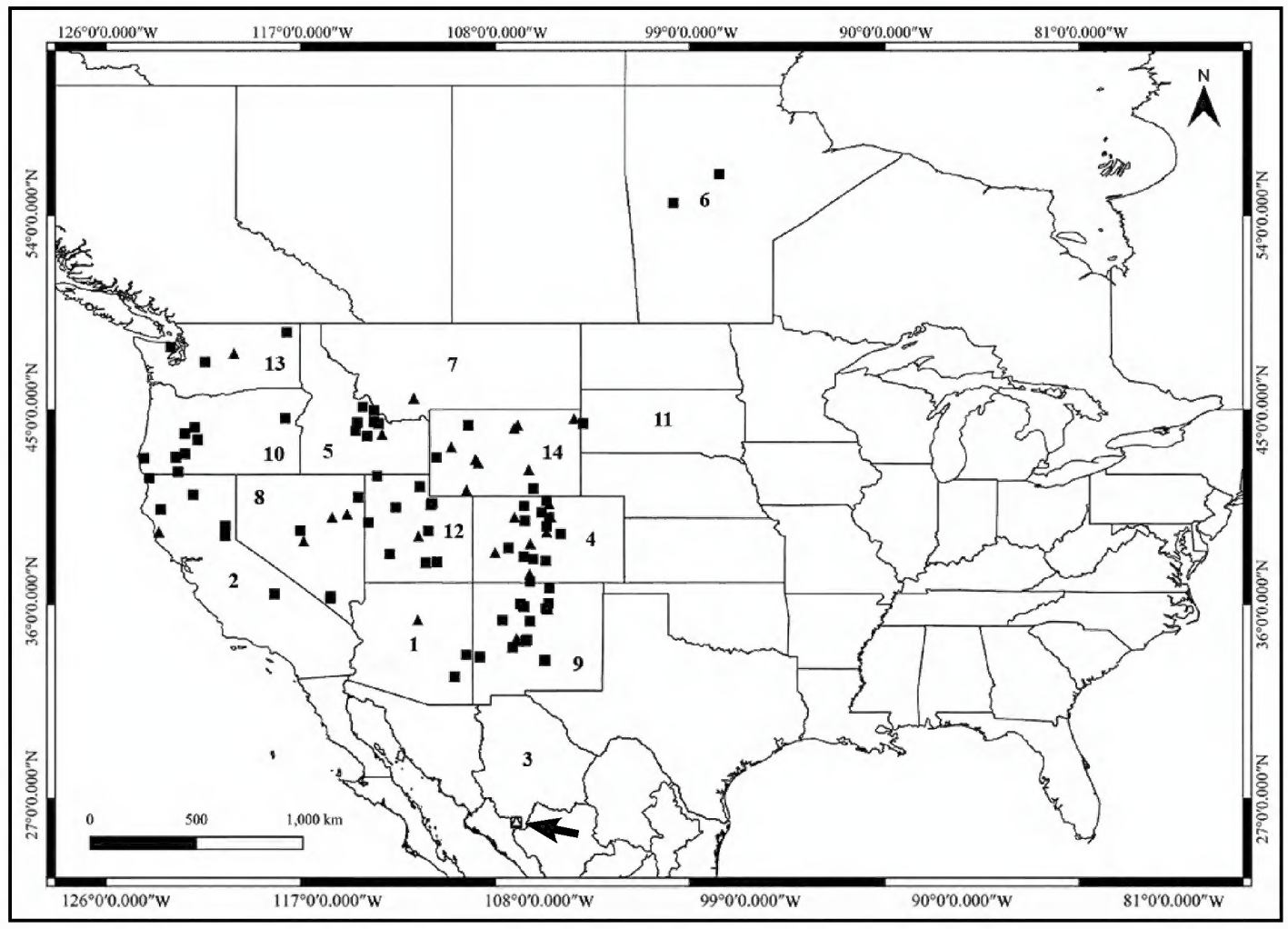


**Figure 3.** Map of part of North America showing the localities were specimens of *Heteroxynema cucullatum* (■) and *Rauschtineria eutamii* (▲) have been collected (1 Arizona; 2 California; 4 Colorado; 5 Idaho; 6 Manitoba; 7 Montana; 8 Nevada; 9 New Mexico; 10 Oregon; 11 South Dakota; 12 Utah; 13 Washington; 14 Wyoming). Data source: https://arctos.database.museum/). The new collection location in México (3 Chihuahua) is indicated by □ and △, respectively (and pointed to by an arrow).

Both species parasitize the intestinal caecum of several *Neotamias* species which occur in sympatry primarily throughout the Pacific and Mountain regions of the United States (Hall 1916; Hugot 1980; Bell et al. 2015, 2016, 2018). The distribution of both species of nematodes reflects the distributions of its hosts (Figure 3 and Appendix 1). *Rauschtineria eutamii* has been documented in 10 host species, while *H. cucullatum* is known to have 16 host species. Nine species of chipmunks host both pinworm species (Bell et al. 2015), and one of them is *Neotamias dorsalis*, which is common in many habitats, from salt forests to pine forests, in parts of Arizona, Colorado, Idaho, Nevada, New Mexico, Utah, and Wyoming in the United States, as well as in Chihuahua, Coahuila, Durango, and Sonora in northern Mexico (Castillo 2014; Lacher et al. 2016). *Heteroxynema cucullatum* has been recorded parasitizing *N. dorsalis* in Oregon and Wyoming (Bell 2016) and *R. eutamii* in Utah (Fransen and Grundman 1961; Bell et al. 2018). This is the first time both species of oxyuroids have been recorded in *N. dorsalis* in Mexico.

The life cycle of the two nematode species presented here is monoxenous. We suggest that the transmission of these organisms between host individuals may occur through caecotrophy, a behavior in which animals eat fecal material for its nutritive value. Rodent feces (droppings) are released as mucus-coated oval balls, which are rich in water, protein, and B vitamins (Iturbe-Morgado et al. 2017).

These nematodes are capable of parasitizing a few closely related host species. This relationship is explained based on the proposal that *H. cucullatum* infected the ancestor of the genus *Neotamias*, while *R. eutamii* colonized species of the same genus approximately 2.5 million years ago. This ancestral relationship, along with relative divergences within the parasite lineages and events of codivergence with hosts, has allowed the relationship between pinworms and chipmunks to persist over time (Bell et al. 2018).

Cerro Mohinora exhibits phytogeographic affinities with the herbaceous-dominated mountain plant communities of the southern Rocky Mountains, where the genus *Neotamias* occurs. Mohinora is considered a Pleistocene refugium (Martínez 1991; McDonald et al. 2011; Bell et al. 2018; Acosta et al. 2020), and, during the Pleistocene, the relationship between nematodes and *Neotamias* had already existed. The fauna in Cerro Mohinora has affinities with that in forests above 3,000 m elevation in Arizona and New Mexico, where *N. dorsalis* also occurs (Bell et al. 2018).

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## ADDITIONAL INFORMATION

### Conflict of interest

The authors declare that no competing interests exist.

### Ethical statement

No ethical statement is reported.

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This study was granted by Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) through the Project PJ006 granted to J. A. Fernández.

### Author contributions

Conceptualization: JAF, JFO. Investigation: MFAC, JFO. Resources: JAF. Visualization: JFO. Writing – original draft: JFO. Writing – review and editing: JFO, MFAC, JAF.

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### Data availability

All data that support the findings of this study are available in the main text.

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## APPENDIX 1

List of hosts parasitized by *Heteroxynema cucullatum* and *Rauschtineria eutamii*, corresponding to the states (US and Mexico) and provinces (Canada) in Figure 3. The data were obtained from https://arctos.database.museum/.

***H. cucullatum*.** 1 Arizona: *Neotamias cinereicollis* and *N. dorsalis*. 2 California: *Neotamias* sp. *N. senex*, *N. siskiyou*, *N. sonomae* and *N. speciosus*. 4 Colorado: *N. amoenus*, *N. minimus*, *N. quadrivittatus*, *N. rufus* and *N. umbrinus*. 5 Idaho: *Neotamias* sp. and *N. amoenus*, 6 Manitoba: *N. minimus*. 8 Nevada: *N. dorsalis*, *N. palmeri*, *N. panamintinus* and *N. umbrinus*. 9 New Mexico: *N. canipes*, *N. cinereicollis*, *N. dorsalis*, *N. minimus* and *N. quadrivittatus* 10 Oregon: *N. amoenus*, *N. siskiyou* and *N. townsendii*. 11South Dakota: *N. minimus*. 12 Utah: *N. dorsalis* and *N. umbrinus*. 13 Washington: *N. ruficaudus* and *N. townsendii*. 14 Wyoming: *N. amoenus*, *N. minimus* and *N. umbrinus*.

***R. eutamii*.** 1 Arizona: *N. dorsalis*. 2 California: *N. alpinus*, *N. ochrogenys N. sonomae* and *N. speciosus*. 4 Colorado: *N. minimus*, *N. quadrivittatus*, *N. rufus* and *N. umbrinus*. 5 Idaho *N. amoenus*. 7 Montana: *N. amoenus*. 8 Nevada: *N. dorsalis*, *N. minimus* and *N. umbrinus*. 9 New Mexico: *N. canipes*, *N. cinereicollis*, *N. dorsalis*, *N. minimus* and *N. quadrivittatus*. 10 Oregon: *N. siskiyou*. 11 South Dakota *N. minimus*. 12 Utah: *N. dorsalis*, *N. rufus* and *N. umbrinus*. 13 Washington: *N. amoenus*. 14 Wyoming: *N. minimus* and *N. umbrinus*.